Pexa-Vec double agent engineered vaccinia: oncolytic and active immunotherapeutic
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Oncolytic immunotherapies (OI) selectively infect, amplify within and destroy cancer cells, thereby representing a novel class of anti-cancer therapy. In addition to this primary mechanism-of-action (MOA), OI based on vaccinia have been shown to selectively target tumor-associated vasculature, triggering an acute reduction in tumor perfusion. This review focuses on a third complementary MOA for this product class: the induction of active immunotherapy. While the active immunotherapy approach has been validated by recent product approvals, the field is still faced with significant challenges. Tumors have evolved diverse mechanisms to hide from immune-mediated destruction. Here we hypothesize that oncolytic immunotherapy replication within tumors may tip the immune balance to allow for the effective induction and execution of adaptive anti-tumor immunity, resulting in long-term tumor control following OI clearance. This immune activation against the cancer can be augmented through OI ‘arming’ for the expression of immunostimulatory transgene products from the virus genome. With the first vaccinia OI (Pexa-Vec, thymidine kinase-inactivated vaccinia expressing Granulocyte-colony stimulating factor [GM-CSF]) now in advanced-stage clinical trials, it has become more important than ever to understand the complimentary MOA that contributes to tumor destruction and control in patients.

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An introduction to Pexa-Vec oncolytic immunotherapy
Therapeutic oncolytic immunotherapies (OI) constitute a class of cancer-targeted products that have unique mechanisms-of-action (MOA) compared with approved cancer therapeutics. OI are replication-competent viruses designed for selective replication and amplification within tumor cells [1,2]. These agents can also stimulate anti-cancer immunity directly (active immunotherapy), presumably through induction of intratumoral immune danger signals coupled with release of tumor antigens (discussed in more detail below). Numerous biological properties of vaccinia and other poxviruses have been proposed as optimal for their development as oncolytic and immunostimulatory agents for cancer [3⁴–9]. First, their potency is high relative to other virus species due to their rapid replication, cell lysis and motile spread; the rate of viral spread is a critical factor in determining oncolytic virus efficacy [10]. In addition, poxviruses have extremely broad tumor tissue infectivity [11]. Poxviruses are also highly efficient at spreading through the blood to distant tumors (including metastases) within a host, while maintaining resistance to neutralization within the blood stream. The result is that systemic delivery and spread between tumors is highly efficient with vaccinia [11–13]. Finally, the relatively large transgene expressing capacity (25–50 kB) allows the expression of multiple therapeutic and monitoring transgenes [3⁴,14]. Indeed, vaccinia has been explored clinically as a tumor-antigen delivery vector for cancer vaccination against a variety of cancer types including melanoma, cervical cancer, renal cell carcinoma, colorectal cancer, prostate cancer, and non-small cell lung cancer [15] (reviewed extensively in [16]).

Pexa-Vec (pexastimogene devacirepvec, JX-594) is an oncolytic immunotherapy (OI) based on the Wyeth vaccinia vaccine strain which has been engineered for viral thymidine kinase (TK) gene inactivation, and expression of the human granulocyte-monocyte colony stimulating factor (hGM-CSF) and β-galactosidase (β-gal) transgenes under control of the synthetic early-late and p7.5 promoters, respectively [12,17]. Administration of GM-CSF protein has long been used in patients to stimulate white blood cell (WBC) counts and has a long track record in cancer vaccination via expression in tumor cells or by viral vector delivery [18–20]. GM-CSF expression and subsequent induction of GM-CSF responsive WBC subsets has been detected in patients treated with Pexa-Vec by intravenous or intratumoral injection [21,22,23]. Over 300 patients with advanced cancer have been treated with Pexa-Vec to date on Phase 1, 2 and 2b clinical trials ([21,22,24–26]
and unpublished data). In a Phase 1 trial of intratumoral injection into liver tumors, Pexa-Vec was well-tolerated and associated with virus replication, expression of biologically active GM-CSF and tumor necrosis [21]. In a subsequent Phase 1 trial of intravenous administration, Pexa-Vec was detected in a dose-related fashion both by immunohistochemistry and quantitative polymerase chain reaction (Q-PCR) in tumor biopsy samples collected one week following infusion [22]. Intravenous Pexa-Vec treatment was well-tolerated, with transient mild to moderate flulike symptoms being the most common adverse events [21,22]. Anti-tumor effects were observed in advanced cancer patients on these early-phase clinical trials [17,21,22,27,28]. A randomized Phase 2 dose-ranging study in patients with advanced hepatocellular carcinoma (HCC; primary liver cancer) (n = 30) demonstrated that intratumoral Pexa-Vec injection was well-tolerated. Further, overall survival was significantly longer in the high-dose arm compared with the low-dose arm (median 14.1 months versus 6.7 months, hazard ratio 0.39; p-value 0.020) [24]. In contrast, a Phase 2b clinical trial in HCC patients who failed sorafenib therapy (n = 120) was recently completed and did not achieve the primary endpoint of prolonging overall survival in Pexa-Vec treated patients when compared to patients treated with best supportive care in this last-line, poor prognosis patient population. A Phase 3 study of Pexa-Vec in first-line HCC patients is planned.

**Challenges with current active immunotherapy approaches: hurdles to overcome**

Despite recent product approvals, hurdles remain for active immunotherapy as a field. Spontaneous, naturally-occurring cancers co-evolve with the host immune system. The immune system selects for the outgrowth of tumor cells that have low antigenicity. In addition, a micro-environment is selected for that is conducive to immune evasion and active immune suppression. At the same time, the immune system is also shaped by the tumor. The repertoire of potentially tumor-reactive T cell clones is often tolerated or anergized against tumor antigens. As a result, potentially responsive clones that could be activated, even in the absence of such immune suppressive mechanisms, are functionally inert. Thus, an ‘awakening’ intervention is required [29]. The mechanisms of T cell tolerance induction are beginning to be understood, and both central and peripheral tolerance are involved. Central tolerance involves the shaping of the immune repertoire to avoid self-recognition by pre-deletion of T cell progenitors that are autoreactive, thus restricting the number of potentially tumor-reactive naïve T cells. In addition, powerful peripheral tolerance mechanisms are in play to prevent the erroneous activation of potentially autoreactive T cell clones [30,31]. These blockades in the tumor microenvironment (reviewed extensively in [32]) include the following: (1) low expression of costimulatory molecules and/or major histocompatibility complex (MHC) on tumor cells; (2) enhanced expression of T cell inhibitory molecules such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Programmed Death-1 (PD-1); (3) production of soluble immune suppressive factors such as indoleamine 2,3-dioxygenase (IDO), transforming growth factor-β (TGF-β), interleukin 10 (IL-10), vascular endothelial growth factor (VEGF); (4) regulatory T cells (Treg); and (5) myeloid-derived suppressor cells (MDSC). A large portion of the responsibility for maintaining peripheral tolerance rests on dendritic cells (DCs) and their ongoing sampling of self-antigen. Hypothetically, any intervention that reverses or overcomes any of these immunosuppressive forces would favor recruitment of effector cells and would facilitate elicitation of their effector function. In fact, investigators have attempted to prime and recruit tumor-reactive cytotoxic T lymphocytes (CTL) by inducing local inflammation using various Toll-like receptor (TLR) ligands including lipopolysaccharide (LPS), CpG DNA, or imiquimod, achieving signs of tumor regression [33–36]. While promising, systemic application of such potent inflammatory agents is not feasible, and such approaches are thus limited to cancers that are amenable to local injection. If such inflammation could be delivered systemically, and then amplified locally in tumors selectively, this therapeutic approach would be potentially viable.

Thus, efforts to mount immune responses without modifying the potent immune suppression within the tumor microenvironment are largely inadequate. In addition, classical cancer vaccines are limited by the relevant tumor antigen(s) choice, penetrance, and potential for mutation or evolved loss of expression. Generation of potent systemic anti-tumor immunity does not necessarily translate into tumor infiltration and effective activity of primed CD8+ T cells [37]. Even transplantation of highly expanded and activated tumor-reactive tumor infiltrating lymphocytes (TILs) are met with the challenges of tumor infiltration and immune suppressive forces within the tumor, as well as host homeostatic mechanisms that curtail the longevity and further expansion of adoptively transferred T cells. For maximal efficacy, an immunotherapeutic approach should prime endogenous tumor antigen-reactive T cells, concomitantly recruit tumor antigen-reactive T cells into the tumor, and finally reverse the immunosuppressive tumor milieu. An immunotherapeutic strategy that exhibits these effects, in combination with a therapy that debulks tumors without immunosuppression, should maximize clinical benefit.
The GM-CSF-armed OI Pexa-Vec as active immunotherapy

Tipping the immune balance

Pexa-Vec infection of tumor cells can provide (1) TLR stimulation, (2) DC activation and (3) an abundance of tumor antigen available for cross-presentation by DCs following uptake of dying cancer cells. Specifically, vaccinia virus infection activates antigen presenting cells (APCs) via TLRs 2 and 8 [38–41], leading to secretion of type I interferon (IFN), IL-1, IL-6 and IL-12, activation of natural killer cells, and CTL expansion. In addition, GM-CSF transgene expression from Pexa-Vec results in WBC expansion, DC activation and maturation. Therefore, oncolytic cell death should result in TLR and DC stimulation in conjunction with an ample supply of dying tumor cells (tumor antigens). This combination of stimulus plus endogenous tumor antigen should prime naïve tumor-reactive cells and awaken anergic/tolerized clones in the circulation. As mentioned previously, priming a tumor-reactive CTL response is only part of the battle. Enticing these cells to traffic to, take up residence in, and mount an attack against the tumor is a largely underestimated challenge. Due to their selective replication within tumors — coupled with the ability to be delivered to tumors systemically in patients [22*] — Pexa-Vec offers the opportunity for systemic delivery of an inflammatory stimulus to the tumor [42]. The pro-inflammatory signals at the site of the tumor microenvironment may override the suppressive action of Treg and other immunosuppressive factors, thereby ‘tipping the balance’ in favor of activating naïve T cells (overcoming immunosuppression). As an example, expression of cytokines such as IL-6 locally may alleviate Treg suppression of anti-tumor immune responses [43]. Furthermore, cytokine production at the tumor site can result in activation of the local endothelium and increased vascular permeability, promoting recruitment, adhesion, and infiltration of immune cells into the tumor. Finally, the very nature of tumor-targeted cell death (oncolysis), OI is a debulking therapy.

An overview of the hypothesis outlining the multi-faceted immunotherapeutic MOA of the OI Pexa-Vec is represented in Figure 1.

Support for immunotherapeutic potential of oncolytic vaccinia/Pexa-Vec

Vaccinia has had a long history of use as a vaccine vector in the setting of infectious diseases. Vaccinia was first used in the smallpox eradication campaign (reviewed in [44]), as well as for vaccination against a variety of infectious diseases including rabies, malaria, hepatitis C virus (HCV), human immunodeficiency virus (HIV) and tuberculosis (reviewed in [16]). Vaccinia has also been explored clinically as a tumor-antigen delivery vector for cancer vaccination [16].

Confirmation that a replicating oncolytic vaccinia virus could induce a potent anti-tumor response was reported by Yang et al. [43]. Immune stimulation provided by a replicating vaccinia vaccine vector could both prime a de novo response, as well as break tolerance to a self-antigen; this contrasted with the failure of a mature DC vaccine vector in the same model. Given that most tumor antigens are self-antigens, such a potent immune stimulus may be necessary to achieve fulminant immune reactivity to a self/tumor antigen. Breaking tolerance in this model required persistent TLR activation by vaccinia; DCs expressing the self-antigen could not provide this. In this paper, immunity was achieved against a self-antigen expressed by tumor cells, and only immunization with the model antigen-expressing vaccinia virus was capable of rejecting tumor challenge. Whether this immunity could reject an established tumor, and therefore raise effective therapeutic immunity in the context of an immunosuppressive environment, was not investigated.

Intravenous therapy with an oncolytic vaccinia virus in human xenograft models revealed infiltration of MHC class II-positive cells and induction of pro-inflammatory cytokines [45].

In addition, Thorne et al. studied induction of immune cell infiltration (CD4+ T cells, CD8+ T cells, natural killer cells, macrophages) in the context of oncolytic vaccinia therapy. In particular, they observed enhanced immune infiltration into tumors in animals that had been pre-vaccinated with vaccinia, likely triggering an exaggerated immune response versus the viral vector, with potential ‘collateral damage’ to the tumor tissue [42]. Further preclinical and clinical studies are necessary to study the detailed mechanisms of antitumor immune induction of oncolytic vaccinia vectors, including Pexa-Vec.

To build on this potential for immunization with the vaccinia vector backbone, immunostimulatory cytokines can be encoded in the vector (e.g. GM-CSF in the case of Pexa-Vec). In fact, the original intent of the design of Pexa-Vec was the local delivery, expression and amplification of GM-CSF for in situ vaccination alone [17]. In a rabbit model, Pexa-Vec treatment resulted in increased infiltration of CD4+ and CD8+ T cells when compared to phosphate buffered saline (PBS) treated control rabbits [12]. Furthermore, in the immunocompetent murine CT26 tumor model we observed that inclusion of murine GM-CSF transgene in the Pexa-Vec backbone produced a superior therapeutic benefit when compared to the Pexa-Vec expressing human GM-CSF (human GM-CSF is not active in mice) (unpublished data). Similarly, Pexa-Vec expressing murine GM-CSF exhibited superior anti-tumor efficacy in a murine glioma model, although the potential for inflammation in the brain in this setting
Model of Pexa-Vec as a multi-faceted cancer immunotherapeutic. (a) Antigen presenting cells (APCs — dendritic cells depicted here) collect antigens at the site of the tumor and carry their cargo to regional draining lymph nodes where they interact with naïve T lymphocytes. In the absence of oncolytic poxvirus infection (no danger signal), these T cells are anergized and do not develop anti-tumor effector function. Following viral infection of the tumor, Pexa-Vec rapidly kills tumor cells by direct oncolysis or indirectly by bystander killing mechanisms, acting as a debulking therapy and supplying abundant tumor antigen for uptake and cross-presentation by resident DCs. These DCs mature and prime
of an intracranial tumor was shown [46]. Improved efficacy upon incorporation of GM-CSF was also observed in an oncolytic vaccinia vector based on Western Reserve vaccinia (vvDD; a bioselected version of the Wyeth vaccine strain with deletion of thymidine kinase and vaccinia growth factor [47]). JX-963, a version of vvDD expressing GM-CSF exhibited superior efficacy to vvDD control in a rabbit tumor model [11]: decreased primary tumor burden, as well as a decreased number of lung metastases, were observed following treatment with JX-963 when compared to the vvDD backbone which does not express GM-CSF.

Pexa-Vec has also been demonstrated to have immune stimulatory properties in patients. Inflammation within tumors was detected following intratumoral Pexa-Vec administration in patients with melanoma [17,26]. Furthermore, functional anti-cancer immunity in the context of Pexa-Vec treatment was demonstrated by measuring induction of antibody-mediated complement-dependent cytotoxicity (CDC) utilizing a panel of tumor cell lines of different histologies [24,48]. Furthermore, T cell responses to β-galactosidase peptides were detected in HCC patients treated with Pexa-Vec, as shown by Enzyme-Linked ImmunoSpot (ELISPOT) analysis; this provides proof-of-concept that T cell responses can be induced to transgenes encoded by oncolytic vaccinia viruses [24].

Finally, additional efficacy upon incorporation of other immunostimulatory cytokines in oncolytic vaccinia backbones has been investigated. Kirn et al. reported protective immunity following tumor eradication with an oncolytic vaccinia virus expressing interferon-β in an immunocompetent tumor model, and that animals were refractory to subsequent tumor challenge [49]. In addition, Lì et al. reported that expression of the chemokine Chemokine (C–C motif) ligand 5 (regulated on activation, normal T cell expressed and secreted; RANTES) resulted in increased lymphocyte chemotaxis to tumors and increased efficacy when compared to the parental vvDD vector [50].

**Conclusion**

OI such as Pexa-Vec represent a novel therapeutic platform with numerous attractive features. Given the good tolerability profile and non-overlapping MOA with existing approved immunotherapeutics, OI such as Pexa-Vec may be applied as part of combination therapy regimens.

**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

4. Expert review article on oncolytic poxviruses.


First demonstration of IV delivery of an oncolytic vaccinia virus.


Comparison of vaccinia based vaccines to dendritic cells vaccines demonstrating persistent TLR signaling triggered by vaccinia generate superior vaccine effects.
